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Cloning and Expression of Mycobacterium Tuberculosis Rv0285 (Pe5) Gene

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Background: PE5 is a member of the PE protein family whose precise function is yet to be understood. There are about 100 members of the PE family proteins in mycobacterium tuberculosis. These glycine- and alanine-rich proteins consist of Proline-Glutamate motifs at their N-termini and may be implicated in pathogenesis of the bacilli.
Objectives: We aimed to clone and over-express the Rv0285 coding region in the BL21 (DE3) *Escherichia coli* strain for the future functional investigations.
Materials and Methods: The PE5 coding region was cloned into a specific vector containing N-terminal GST tag using ligation independent cloning (LIC) method and then the recombinant vector was transferred into the competent TOP10 *E. coli* strain. The positive colonies were screened by the colony PCR approach and finally integration of the constructed expression vector was assessed by DNA sequencing. The vectors were then transferred and expressed in *E. coli* BL21 (DE3) strain and finally the protein expression level was analyzed using SDS-PAGE.
Results: In this study, the PE5 (Rv0285) coding region was amplified as a 309 bp DNA fragment from the mycobacterium tuberculosis H37Rv chromosome using specific sets of primers. The amplicon was then cloned into pLEICS-02 and then confirmed by DNA sequencing. The recombinant protein was over-expressed as a ~ 40 kDa tagged protein in *E. coli*, and finally confirmed by SDS-PAGE analysis.
Conclusions: Our data showed that recombinant PE5 coding region was successfully cloned in pLEICS-02 and expressed in BL21 (DE3) *E. coli* strain as host.

Keywords: Mycobacterium Tuberculosis; Genes

1. Background

Tuberculosis (TB), once thought to be controlled, is now resurgent in many parts of the world, claiming more lives annually than any other infectious disease. Every second someone in the world is newly infected with TB Bacilli and five to ten percent of the infected individuals become infectious at some time during their life. An estimated two billion people, about one third of world's population, is currently infected with TB Bacilli, resulting in about two million deaths annually (1, 2). The major burden of TB is borne by developing countries, particularly in Africa and South-East Asia, mainly because of the reduced socio-economic status, lack of proper public healthcare system, emergence of both multi-drug resistant (MDR) and extensively drug resistant (XDR) tuberculosis and spread of HIV/AIDS (3). However, in economically favored countries with outstanding clinical facilities and proper public healthcare system, other risk factors such as, stressful life, alcohol consumption and particularly increased number of immigrants from high burden counties have resulted in an increase in the number of reported TB cases (2, 4).

The *M. tuberculosis* genome was sequenced completely about 14 years ago (5). The singular GC rich circular chromosome consists of 4.4 Mbp and contains 4006 functional protein genes (6). About 52% of these proteins have been assigned a precise or predicted function (7). An unusual characteristic of the genome is the presence of 167 genes encoding the two unrelated, acidic protein families, known as the PE and PPE proteins (8). These two protein families are recognized by their N-terminal proline-glutamic acid and proline-proline-glutamic acid motifs and it has been suggested that at least some of these proteins are surface exposed and play a role in adhesion, immune modulation and antigenic variation (9, 10). PE5, a member of the PE family proteins, is encoded by the RV0285 gene (11). This 306 bp coding region encodes a polypeptide, containing 102 amino acids (12). Given the possibility that this polypeptide acts as virulence factor, we cloned and then expressed the Rv0285 coding region in the BL21 (DE3) *E. coli* strain for the future functional investigations.

Implication for health policy/practice/research/medical education:
It helps to clone and over-express the Rv0285 coding region in the BL21 (DE3) Escherichia coli strain for the future functional investigations.